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AS AD No. 453398

# TECHNICAL MANUSCRIPT 156

## DEUTERIUM-TOLERANT PASTEURELLA TULARENSIS

OCTOBER 1964

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TECHNICAL MANUSCRIPT 156

DEUTERIUM-TOLERANT PASTEURELLA TULARENSIS

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Project DA012501B02801

October 1964

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### ABSTRACT

Substitution of deuterium oxide for water in liquid medium has been shown to affect the growth and viability of Pasteurella tularensis SCHU S4 adversely as the  $D_2O$  concentration is increased. Prolonged lag phase, cell enlargement, chain formation, and clumping were observed. This report concerns the selection and characterization of a deuterium-tolerant mutant (SCHU DT), variation between strains with regard to growth on solid medium containing  $D_2O$ , and properties of organisms grown in the presence of deuterium. SCHU DT was capable of multiplication on solid medium containing 98 per cent  $D_2O$  and showed no reversion when serially transferred more than 20 times on medium without  $D_2O$ ; it possessed aerosol properties similar to parent SCHU S4, but was of significantly lower virulence for the mouse, guinea pig, and rabbit. The majority of guinea pigs surviving a respiratory exposure of  $10^4$  SCHU DT were at least as resistant to challenge with SCHU S4 as animals administered live vaccine strain LVS. Several Eastern European and Asiatic strains were able to multiply in medium containing 98 per cent  $D_2O$  whereas strain SCHU S4 and several SCHU S4 colony-type mutants of lowered virulence were inhibited. Deuterated LVS cells, although less virulent than normal cells, were comparable in immunogenicity for the mouse.

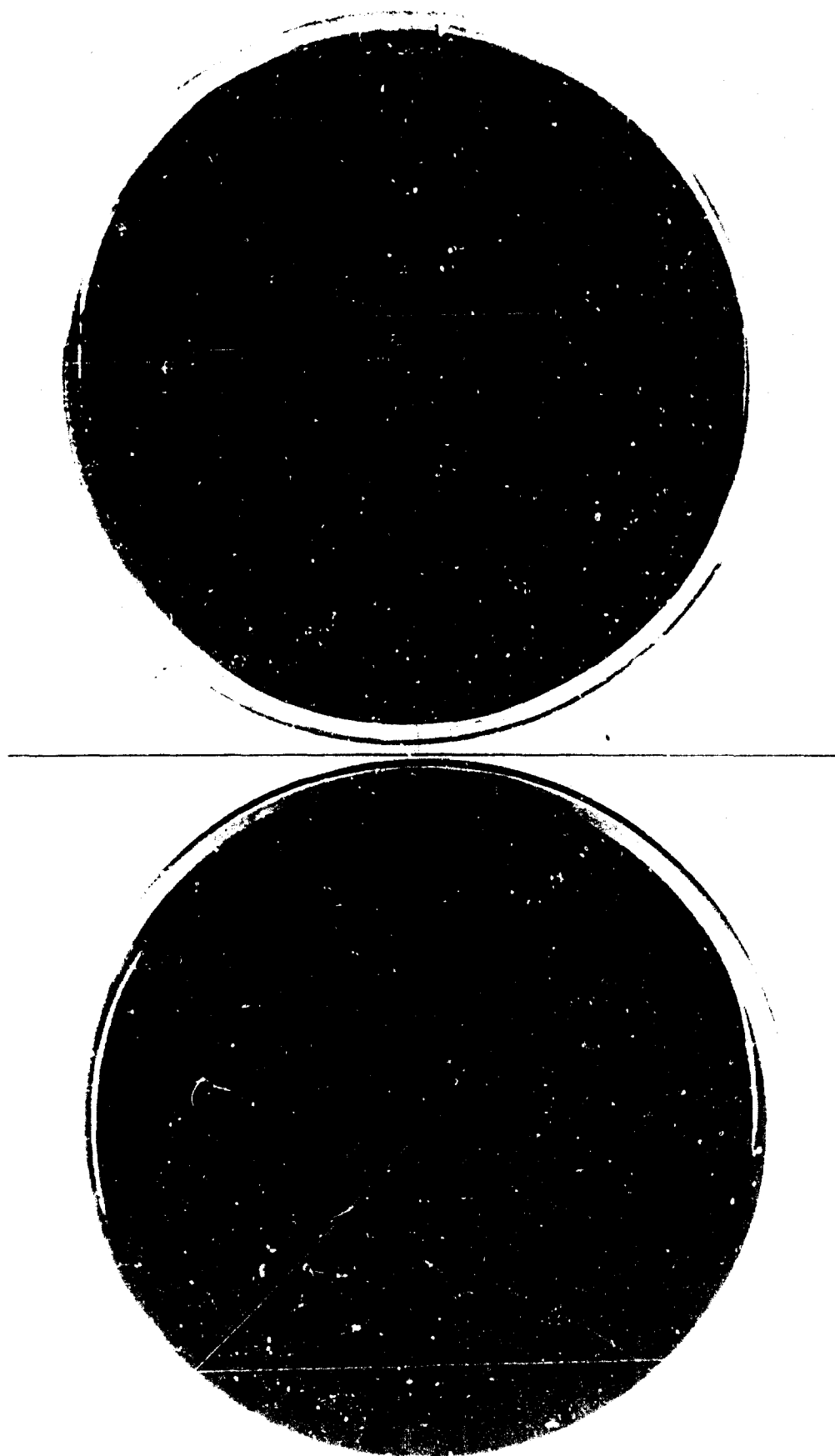


Figure 1. Growth of *P. tularensis* SCHU S4 on Glucose Cysteine Blood Agar Plates Prepared with D<sub>2</sub>O (left) or with H<sub>2</sub>O (right).

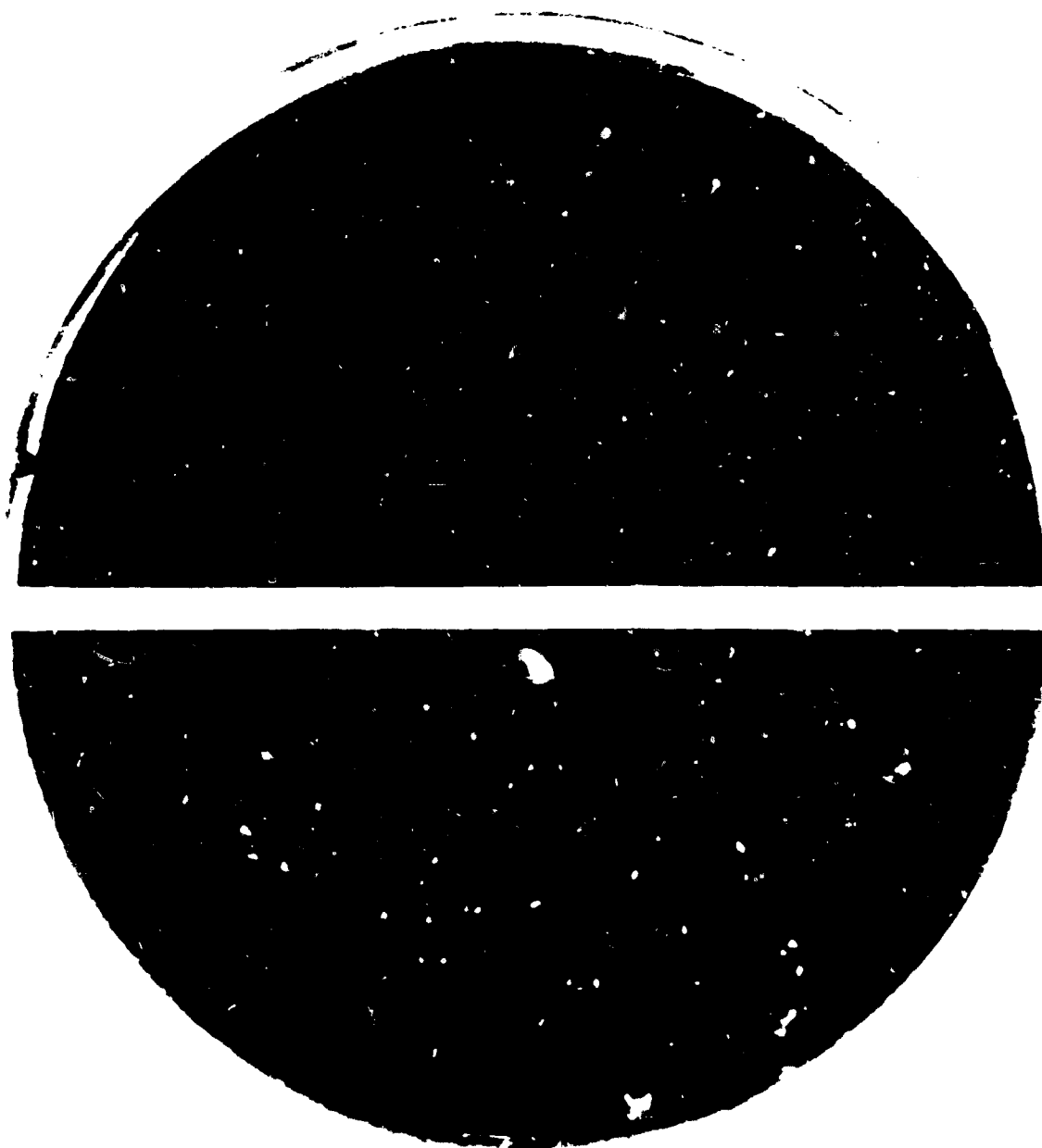


Figure 2. Colonies of Deuterium Tolerant Mutant of *P. tularensis* after 96 Hours on H<sub>2</sub>O Medium (left) and D<sub>2</sub>O Medium (right).

TABLE I. GROWTH OF *PASTEURELLA TULARENSIS* DEUTERIUM-TOLERANT  
MUTANTS ON SOLID MEDIUM

Isolate	No. Colonies from Indicated Dilution			
	0% D <sub>2</sub> O		98% D <sub>2</sub> O	
	10 <sup>-6</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-7</sup>
SCHU DT A	264	31	350	32
SCHU DT B	288	28	316	30
SCHU DT C	564	52	533	55
SCHU S4	528	48	0	0

In Table II data are presented on the subcutaneous virulence of mutant SCHU DT and of the parent SCHU S4 for the mouse, guinea pig, and rabbit. In comparison with strain SCHU S4, the subcutaneous virulence of SCHU DT was considerably lower for all three of the experimental animals. It is of interest that somewhat greater residual virulence was retained for the guinea pig than for the mouse or rabbit. This observation has been confirmed by additional tests.

Data obtained on the viable aerosol recovery, decay rate, and respiratory guinea pig infectivity of SCHU S4 and SCHU DT are presented in Table III. Significantly higher recovery of viable aerosolized organisms was attained with SCHU DT cultivated in liquid medium containing heavy water; the mean recovery of cultures grown in the presence of D<sub>2</sub>O were 0.963 and 0.840 as compared with 0.469 when the medium did not contain D<sub>2</sub>O. Aerosol decay rates of the cultures were comparable and could not be differentiated by statistical analysis. At the respiratory dose range of 500 to 1000 cells used in this experiment, only SCHU S4 caused fatal infections.

Table IV presents comparative aerosol data for mutant DT and three other low-virulence *P. tularensis* strains: SCHU colony-type mutants S2-3 and S1-11, and live vaccine strain LVS. Under the conditions of this preliminary experiment, aerosol recovery of LVS was appreciably higher than for SCHU S1-11; SCHU DT and SCHU S2-3 gave intermediate recoveries in comparison. Of particular interest was the observation that aerosol doses of 20,000 to 95,000 SCHU DT killed 56 to 60 per cent of the animals whereas only occasional animals died as a result of aerosol exposure to SCHU S2-3 or LVS. The dose of SCHU S1-11 was too low for comparison. These data in



TABLE II. SUBCUTANEOUS VIRULENCE OF PASTEURELLA TULARENSIS SCHU DT AND SCHU S4 FOR LABORATORY ANIMALS

Culture	Dose, number cells	Per Cent Dead Within 15 Days <sup>a/</sup>		
		Mouse	Guinea Pig	Rabbit
SCHU DT	10	0	0	- <sup>b/</sup>
	10 <sup>2</sup> -10 <sup>3</sup>	13	0	0
	10 <sup>4</sup>	7	3	0
	10 <sup>5</sup>	10	33	0
	10 <sup>6</sup>	20	59	-
	10 <sup>7</sup> -10 <sup>8</sup>	-	100	-
SCHU S4	10	100	100	100

a. Forty to 60 animals per group.

b. No animals tested.

TABLE III. VIABLE RECOVERY, DECAY RATE, AND GUINEA PIG INFECTIVITY OF AEROSOLIZED PASTEURELLA TULARENSIS STRAINS SCHU S4 AND SCHU DT

Strain	D <sub>2</sub> O in Medium, per cent	Viable Recovery, per cent	Decay Rate, %/min	Mean Dose	Mortality, per cent
SCHU S4	0	0.506	6.13	964	97
SCHU DT	0	0.469	6.72	729	0
SCHU DT	74	0.963	6.35	944	0
SCHU DT	93	0.840	4.59	550	0

TABLE IV. VIABLE RECOVERY, DECAY RATE, AND GUINEA PIG DEFECTIVITY OF SEVERAL PASTEURELLA TULAPENSIS STRAINS

Strain	Viable Recovery, per cent	Decay Rate, %/min	Mean Doses	Mortality, per cent
SCHU S2-3	2.60	5.70	17,400 80,000	0 4
SCHU S1-11	0.44	6.57	3,200 8,000	2 0
SCHU DT	1.74	5.28	20,000 95,000	56 60
LVS	3.66	5.57	36,800 270, 0	0 8

comparison with previously presented subcutaneous virulence information indicate that an aerosol dose of  $10^4$  to  $10^5$  cells of SCHU DT is comparable to a subcutaneous dose of  $10^6$  cells with regard to producing guinea pig lethality.

In Table V the further comparative respiratory virulence of SCHU DT and LVS are presented along with the level of immunity afforded survivors. Aerogenic doses of  $10^2$ ,  $10^3$ , or  $10^4$  viable LVS were not lethal for the guinea pig whereas  $10^4$  cells of SCHU DT, cultivated on deuterated or non-deuterated medium, resulted in the death of 17 to 38 per cent of the animals. Protection afforded guinea pigs against subcutaneous challenge with 1000 LD<sub>50</sub> of SCHU S4 by the exposure to either SCHU DT preparation was at least as good as that obtained with LVS. In contrast to the lethality appearing at the  $10^4$  dose of DT, we routinely find that higher aerosol doses of LVS give increased protection without innocuous effect.

The agglutinin response of guinea pigs to subcutaneous inoculation of SCHU DT showed that 77 per cent administered  $10^2$  viable cells and 100 per cent given  $10^3$  to  $10^5$  viable cells converted serologically. The sera of all animals bled after inhalation of  $10^4$  cells possessed specific agglutinins.

As previously indicated, growth of SCHU S4 plated on solid medium containing 98 per cent D<sub>2</sub>O is inhibited; approximately one in 10,000 cells forms a colony. The spontaneous mutation rate with a frequency of  $10^{-4}$

TABLE V. VIRULENCE AND IMMUNOGENICITY OF PASTEURELLA TULARENSIS STRAINS LVS AND SCHU DT FOR THE GUINEA PIG

Strain	D <sub>2</sub> O in Medium, per cent	Aerosol Dose	Virulence, per cent dead	Immunogenicity, <sup>a</sup> / per cent survivors
LVS	0	10 <sup>2</sup>	0	0
		10 <sup>3</sup>	0	29
		10 <sup>4</sup>	0	38
SCHU DT	0	10 <sup>2</sup>	0	0
		10 <sup>3</sup>	0	13
		10 <sup>4</sup>	17	70
SCHU DT	98	10 <sup>2</sup>	0	0
		10 <sup>3</sup>	0	17
		10 <sup>4</sup>	38	67

a. Challenge dose - 1000 SCHU S4; controls dead within 7 days; 24 animals per group.

represented a one-step mutational event and was similar to the mutation rate for E. coli described by DeGiovanni. Mutants SCHU S4, SCHU S1-11, and SCHU S2-3, were also inhibited by high concentrations of D<sub>2</sub>O. However, as Table VI shows, when strains 503, LVS, or JAP H, all derived from Eastern European or Asiatic cultures, were streaked onto highly deuterated medium, the majority of the cells produced colonies. Colonies on deuterated medium were heterogeneous in size and the time required for their formation was strain dependant; 72 hr for JAP H, 96 hr for LVS, and 120 hr for 503.

Table VII shows data obtained on virulence and immunogenicity of deuterated and nondeuterated LVS for the white mouse. Although subcutaneous virulence of deuterated LVS was lower than nondeuterated (14 versus 40 per cent), protection afforded vaccinated mice against virulent challenge was comparable.

In summary, studies on a stable deuterium-tolerant mutant indicated that deuterium tolerance was associated with a concomitant reduction in virulence for experimental animals. This mutant, closely related to SCHU S4, might be of value in the search for an improved live prophylactic vaccine if not too reactive in more resistant animals. Several Eastern European or Asiatic strains were able to multiply in medium containing 98 per cent D<sub>2</sub>O whereas strain SCHU S4 as well as several SCHU mutants of lowered virulence were inhibited.

TABLE VI. GROWTH OF PASTEURELLA TULARENSIS STRAINS OF GRADED VIRULENCE ON SOLID MEDIUM CONTAINING D<sub>2</sub>O

Strain	No. Colonies from Indicated Dilution					
	0% D <sub>2</sub> O		98% D <sub>2</sub> O			
	10 <sup>-1</sup>	10 <sup>-2</sup>	10 <sup>-4</sup>	10 <sup>-5</sup>	10 <sup>-7</sup>	10 <sup>-8</sup>
SCHU S4	648	60	70 <sup>a</sup> /	0	0	0
SCHU S2-3	453	43	32 <sup>a</sup> /	0	0	0
SCHU-1	490	51	44 <sup>a</sup> /	0	0	0
SCHU S1-11	418	46	10 <sup>a</sup> /	0	0	0
SCHU DT	636	68		TNTC <sup>b</sup> /	652	70
503	500	52		TNTC	360 <sup>a</sup> /	24 <sup>a</sup> /
JAP H	618	63		TNTC	568 <sup>a</sup> /	55 <sup>a</sup> /
LVS	512	70		TNTC	440 <sup>a</sup> /	43 <sup>a</sup> /

a. Heterogeneous colony size.

b. Colonies too numerous to count.

TABLE VII. VIRULENCE AND IMMUNOGENICITY OF DEUTERATED AND NONDEUTERATED LVS FOR THE MOUSE

Vaccine	Virulence, per cent dead	Immunogenicity, per cent survivors day after challenge <sup>a</sup> /	
		10	14
Nondeuterated	40	78	65
Deuterated	14	82	70

a. Challenge dose - 1070 SCHU S4; controls dead within 7 days.